Highlights

- *L. monocytogenes* was reduced by 1-log unit in presence of *P. graminis* CPA-7
- No effect of CPA-7 was observed against *S. enterica*
- SSC and TA of fresh-cut pears was not negatively affected by CPA-7 nor $CaCl_2$ treatment
- Ethanol and acetaldehyde increased during shelf-life regardless of CPA-7 presence
- CPA-7 affected the volatile profile of fresh-cut pears

Evaluation of biocontrol capacity of *Pseudomonas graminis* CPA-7 against foodborne pathogens on fresh-cut pear and its effect on fruit volatile compounds

María Belén Iglesias[1], María Luisa López[1], Gemma Echeverría[2], Inmaculada Viñas[1], Lorena Zudaire[2], Maribel Abadias[2*]

[1]*Food Technology Department, University of Lleida, XaRTA-Postharvest, Agrotecnio Center, Lleida, Spain*

[2]*Institute of Agrifood Research and Technology (IRTA), XaRTA-Postharvest, Lleida, Spain*

*corresponding author: isabel.abadias@irta.cat; T: +34 902789 449 Ext. 1502

ABSTRACT

The application of microorganisms to control the growth of foodborne pathogens is an alternative to the use of chemical additives. In this work, *Pseudomonas graminis* CPA-7 was tested as a biocontrol agent against *Salmonella enterica* and *Listeria monocytogenes* on fresh-cut pear under conditions that simulate its commercial application at 5 ± 1 °C (under a modified atmosphere and antioxidant solution). The quality of the fresh-cut fruit, including the ethanol and acetaldehyde contents and the volatile profile, was determined. After the storage period, the *L. monocytogenes* population was reduced by 1-log unit by the presence of CPA-7; however, CPA-7 was not found to have antagonistic activity against *S. enterica*. The fruit quality (total soluble solids content and titratable acidity) was not negatively affected by CPA-7. The ethanol and acetaldehyde contents increased during the shelf-life of the fruit regardless of the presence of CPA-7. Some volatile compounds were key factors for discriminating samples from the two groups (the control group and the group that was inoculated with CPA-7). Some components are common in the volatile profile of pear (methyl acetate, 3-methylbutyl acetate, 1-butanol, 1-hexanol, and hexanal), and thus increases in their contents could enhance consumers flavour perception.

Keywords: *Listeria*, *Salmonella*, ethanol, acetaldehyde, antagonist

1. Introduction

The consumption of fruits and vegetables provides us with a large amount of micronutrients; therefore, they are basic components of a healthy diet. Many studies have reported that the intake of fruits and vegetables reduces the risk of mortality due to cancer and cardiovascular diseases (Wang et al., 2014). Therefore, the production of fresh-cut fruits and vegetables is increasing because of their health benefits as well as their convenience for consumers.

Minimal fruit and vegetable processing consists of washing, trimming, peeling, cutting or shredding, sanitizing and packing. However, these operations do not guarantee the total elimination of spoilage and foodborne pathogenic microorganisms that could be present in the produce. Several outbreaks associated with the consumption of fresh-cut produce have been reported in recent years (CDC, 2016). Chemical sanitizers and additives are used to preserve fresh-cut produce; however, consumer's concerns regarding these substances in food has promoted the development of alternative techniques.

One such method is biopreservation or biological control. Non-pathogenic microorganisms have been proposed as biocontrol agents. They control the growth of spoilage and pathogenic microorganisms by competing for nutrients or physical space or by producing substances that negatively affect pathogens (Parish et al., 2003). Moreover, some lactic acid bacteria (LAB) have also been studied as biocontrol agents. For example, *Lactobacillus rhamnosus* GG has been reported to control the growth of foodborne pathogens on fresh-cut apple (Alegre et al., 2011) and on fresh-cut pear (Iglesias et al., 2017) and *Lactobacillus plantarum* CIT3 on minimally processed apple (Siroli et al., 2015b). The native microbiota present in fruits and vegetables have also shown antagonistic activity against foodborne pathogens. Leverentz et al. (2006) reported that *Candida* spp., *Discosphaerina fagi*, *Gluconobacter assai* and *Metschnikowia pulcherrima* controlled *L. monocytogenes* and *Salmonella* growth at 10 and 25 °C on fresh-cut apple. Trias et al. (2008) showed that some *Leuconostoc* strains

have bactericidal effects against *L. monocytogenes* and reduced the growth of *Escherichia coli* and *Salmonella typhimurium* on fresh-cut apple at 25 °C. *Pseudomonas graminis* CPA-7, isolated from the surface of an apple, has shown activity against foodborne pathogens on fresh-cut apple and peach (Alegre et al., 2013b) and on fresh-cut apple and melon under conditions simulating commercial applications (Abadias et al., 2014; Alegre et al., 2013a). Recently, Iglesias et al. (2018) demonstrated that this biocontrol agent is also effective on fresh-cut pear. Among the many requirements, biopreservation cultures should not impact the quality of the fresh-cut fruit through possible metabolic reactions during bacterial growth.

Maintaining the sensorial qualities of minimally processed fruit after processing and during the chain of distribution is very difficult. The shelf-life of cut produce is very limited due to browning of the flesh and the loss of flavour (Conway et al., 2002; Toivonen, 2006; Toivonen and Delaquis, 2006). Some factors including variety, ripeness stage, and the atmosphere and temperature of storage affect shelf-life during postharvest storage following processing. Modified atmosphere packaging (MAP) in combination with refrigeration temperatures is used to preserve fresh-cut produce. Low O_2 and high CO_2 can be used to preserve the quality of minimally processed fruit because they inhibit the bioreactions in fruit tissue that may lead to physiological decay (Rosen and Kader, 1989; Sapers and Miller, 1998). However, that gas composition may initiate fermentative pathways that release metabolites such as ethanol that cause off-flavours (Soliva-Fortuny et al., 2002). Moreover, it is known that although a high CO_2 level can inhibit aerobic spoilage microorganisms, it can also allow pathogen growth (Rodriguez-Aguilera et al., 2009). Therefore, it is necessary to maintain an O_2 concentration that is sufficiently low but also over the fermentation threshold (Lakakul et al., 1999).

Concerning firmness, postharvest calcium dips for whole fruit have been demonstrated to preserve firmness, cell wall structure (Glenn and Poovaiah, 1990), nutritional quality (Goldberg, 1984) and fruit flavour (Ortiz et al., 2009). Similarly, combinations of calcium

treatment (0.5-4 %) with packaging under modified atmospheres and low storage temperature (< 5 °C) are generally effective for extending the shelf-lives of minimally processed products.

The aim of this study was to evaluate the antagonistic effect of CPA-7 against *Salmonella* and *L. monocytogenes* on fresh-cut pear treated with $CaCl_2$ after harvest under conditions simulating commercial applications (under MAP and in presence of an antioxidant solution) at 5 ± 1 °C. In addition, the effect of CPA-7 on some quality parameters, including ethanol and acetaldehyde contents and the volatile profile, were evaluated throughout storage.

2. Materials and Methods

2.1. Bacterial strains and inoculum preparation

As pathogen microorganisms, five serovars of *Salmonella enterica* subsp. *enterica* were used, namely, Agona (ATCC BAA-707), Michigan (ATCC BAA-709), Montevideo (ATCC BAA-710), Gaminara (ATCC BAA-711) and Enteritidis (CECT-4300), along with five serovars of *Listeria monocytogenes*, namely, serovar 1a (CECT 4031), serovar 3a (CECT 933); serovar 4d (CECT 940), serovar 4b (CECT 4032) and serovar 1/2a, which had previously been isolated in our laboratory from a fresh-cut lettuce sample (Abadias et al., 2008). *S. enterica* and *L. monocytogenes* strains were grown individually in tryptone soy broth (TSB, Biokar Diagnostics, France) medium and in TSB supplemented with 6 g L^{-1} of yeast extract (TSBYE), respectively, for 20-24 h at 37 ± 1 °C.

Pseudomonas graminis strain CPA-7 (deposit number CBS 136973, Centraalbureau voor Schimmelcultures, CBS, Utrech, The Netherlands), isolated in our lab from the surface of an apple (Alegre et al., 2013b), was used as antagonist. It was grown in TSB for 20-24 h at 25 ± 1 °C. Bacterial cells were harvested by centrifugation at 9800 x g for 10 min at 10 °C. Afterwards, the pathogen cells were resuspended in saline solution (SS; 8.5 g L^{-1} NaCl), and the CPA-7 cells were suspended in sterile distilled water. A single suspension of the five *S. enterica* serovars and the *L. monocytogenes* serovars was produced by mixing equal volumes of each concentrated suspension.

To inoculate the fruit, an aliquot of each of the concentrated bacterial suspensions was added to an antioxidant solution (2 % ascorbic acid + 2 % sodium citrate + 1 % $CaCl_2$), which was selected based on previous studies (Iglesias et al., 2018), to obtain solutions of approximately 10^5 cfu mL^{-1} in the case of *S. enterica* and *L. monocytogenes* and 10^7 cfu mL^{-1} for CPA-7. Inoculum concentrations were checked by plating appropriate dilutions onto XLD (xylose-lysine-deoxycholate Agar, Biokar Diagnostics, France) for *S. enterica*, onto Palcam agar (Palcam Agar Base with selective supplementation, Biokar Diagnostics, France) for *L. monocytogenes*, and onto tryptone soy agar (TSA, Biokar

Diagnostics, France) for CPA-7. The plates were incubated at 37 ± 1 °C for 24 for *S. enterica*, at 37 ± 1 °C 48 h for *L. monocytogenes*, and at 30 ± 1 °C for 48 h for CPA-7.

2.2. Fruit processing

'Conference' pears (*Pyrus communis* L. cv. Conference) were used in this study. After harvest, the pears were divided in two lots. Whole fruits of lot 1 were dipped in water at 25 °C for 5 min (control group), and whole fruits of lot 2 were dipped in a solution containing 10 g L^{-1} $CaCl_2$ at 25 °C for 5 min. Afterwards, the pears of both lots were stored at 0 ± 1 °C for 5 months in a controlled atmosphere (2 kPa O_2 and 1 kPa CO_2) leading up to the experiment.

After this storage period, the pears were stored at 20 °C until they reached the optimum ripeness stage for processing (44 ± 3.2 N) (Soliva-Fortuny et al., 2004). Prior to the experimental studies, the pears were sanitized by immersion into a 0.1 g L^{-1} NaOCl solution adjusted to pH 6.5 using citric acid and then rinsed and dried. After that, the pears were peeled and cut into 10 wedges using a handheld apple corer and slicer.

2.3. Fruit inoculation and packaging

To carry out the experiment, the following treatments were prepared: (a) control: antioxidant solution; (b) Sal + Lm: antioxidant solution inoculated with *S. enterica* and *L. monocytogenes* at 10^5 cfu mL^{-1}; (c) CPA-7: antioxidant solution with 10^7 cfu mL^{-1} CPA-7 cells; and (d) Sal + Lm + CPA-7: antioxidant solution containing *S. enterica* and *L. monocytogenes* (10^5 cfu mL^{-1}) and CPA-7 (10^7 cfu mL^{-1}). The pear wedges were dipped into these solutions (1:2 w/v) for 2 min in an orbital shaker at 150 rpm on an orbital shaker. After that, the fresh-cut pears were allowed to dry open to air at room temperature. Approximately 120 ± 5 g of pear wedges were placed in 400-mL polyethylene terephthalate ShelfMaster™ Pronto™ trays (PlusPack, Denmark) and sealed with peelable plastic with an O_2 permeability of 180 cm^3 m^{-2} day^{-1} atm^{-1} at 23 °C

(film PET OLAF interior and OPP exterior with a line of holes of 60 - 80 µm each and 75 mm apart from each other). The film used in this study was selected based on the results of previous studies according to the quality parameters of the fresh-cut pear and the survival and efficacy of CPA-7 (Iglesias et al., 2018).

The trays of fresh-cut pears were stored at 5 ± 1 °C. Microorganism populations were determined the day of inoculation and after 2, 6 and 9 days of storage in the three sample trays. The *S. enterica* and *L. monocytogenes* populations were evaluated in treatments (b) and (d), and CPA-7 was evaluated in treatment (c). Total aerobic mesophilic counts (TAM) were determined in control samples (a). For analysis, 10 g of pear from each tray was mixed with 90 mL of buffered peptone water (BPW, Oxoid, LTD, Basingstoke, Hampshire, England) in a sterile bag and homogenized in a masticator (IUL Instruments, Barcelona, Spain) set at 8.5 strokes s^{-1} for 90 s. Serial dilutions were prepared with saline peptone (SP; 8.5 g L^{-1} NaCl and 1 g L^{-1} peptone), and the solutions were plated in duplicate onto Palcam (*L. monocytogenes*), XLD (*S. enterica*) and on Plate Count Agar (Biokar Diagnostics, France). The agar plates were incubated at 37 ± 1 °C for 24 h for *S. enterica*, at 37 ± 1 °C for 48 h for *L. monocytogenes*, at 30 ± 1 °C for 48 h for CPA-7 and at 30 ± 1 °C for 72 h for TAM.

2.5. Determination of the physical and chemical parameters

To determine if the presence of CPA-7 impacts the quality of the fresh-cut pear, quality parameters were measured in treatments (a) and (c) (without foodborne pathogens). Three determinations (one per each tray) per treatment were made.

2.5.1. Headspace gas composition

Before each microbial analysis at each sampling time, the O_2 and CO_2 concentrations inside the trays were measured using a handheld gas analyser (CheckPoint O_2/CO_2, PBI Dansensor, Denmark). An adhesive septum was attached to the film, and a needle was used to determine the gas composition. The results are expressed as kPa.

2.5.2. Measurement of soluble solids content and titratable acidity

At each sampling time, the soluble solids content (SSC) in juice extracted by crushing the pear wedges in a blender was measured at 20 °C with a handheld refractometer (Atago Co. Ltd., Tokyo, Japan). The results are expressed as %.

To measure the titratable acidity (TA), three measurements per treatment were made at each sampling point. Ten millilitres of pear juice was diluted with 10 mL of distilled water, and the solution was titrated with 0.1 N NaOH up to pH 8.2. The results were calculated as g of malic acid per litre of solution.

2.5.3. Ethanol and acetaldehyde headspace concentrations

The contents of ethanol and acetaldehyde were determined according to the protocol described by Echeverría et al. (2004) with slight modifications. These compounds were extracted from the same juice used to determine SSC and TA. Juice samples (5 mL) were stored at −20 °C until analysis. Samples were transferred to a 10-mL test tube with a screw cap and incubated in a water bath at 60 °C. After 60 min, a 1 mL samples of the headspace gas was taken with a syringe and injected into an Agilent Technologies 6890N gas chromatograph (GC) for the determination of both the acetaldehyde and ethanol concentrations. To do this, the gas chromatograph was equipped with a flame ionisation detector (FID) and a column (2 m × 2 mm i.d.) containing 5 % Carbowax on 60/80 Carbopack (Supelco, Bellefonte, PA, USA). The temperature of the injector, detector and oven were 180, 220 and 80 °C, respectively. Tissue concentrations were calculated using ethanol and acetaldehyde calibration curves prepared by measuring the headspace of Milli-Q water spiked with a known amount of ethanol and acetaldehyde at increasing concentrations and are expressed as $\mu L\ L^{-1}$.

2.5.4. Determination of the volatile compounds

Headspace solid-phase microextraction (HS-SPME) was used for the extraction and to determine the concentrations of the volatile compounds. SPME fibres coated with a 65-μm layer of polydimethylsiloxane–divinylbenzene (65 μm PDMS/DVB; Supelco Co., Bellefonte, PA, USA) were used. Fibres were activated before sampling according to the manufacturer's instructions.

Four pieces of fruit per tray (n = 3) for each treatment were cut in small pieces, frozen with liquid N_2, crushed, and immediately transferred to -80 °C storage until the volatile components could be analysed.

For each extraction, 4 g of the homogenized crushed pulp was placed into a 20-mL screw-cap vial containing 0.5 g of NaCl to facilitate the release of volatile compounds. Prior to sealing the vial, 1 μL of 0.086 mg L^{-1} butyl benzene/diethyl ether was added as an internal standard, and the solution was mixed with a glass rod. A magnetic stirrer was added to each vial, and the vials were placed into a constant-temperature water bath at 60 °C with stirring. Samples were equilibrated for 20 min, and then the SPME fibres were exposed to the head space of the sample for 30 min to adsorb the analytes according to the procedure described by Qin et al. (2012). The volatile compounds were subsequently desorbed over 10 min at 240 °C into the splitless injection port of the chromatograph. The volatile constituents were identified and quantified with an HP 5890A gas chromatograph with a flame ionization detector equipped with a capillary column with cross-linked free fatty acids as the stationary phase (FFAP; 50 m × 0.2 mm × 0.33 μm). Helium was used as the carrier gas at a constant flow of 1.0 mL min^{-1}. The injector and detector temperatures were 240 °C. The oven temperature programme was 40 °C for 1 min, increasing at 2.5 °C min^{-1} to 115 °C, then increasing at 8 °C min^{-1} to 225 °C and holding for 15 min. Compounds were identified by comparing their respective retention index with those of standards. All of the standards for the volatile compounds studied in this work were analytical grade or the highest quality available. Quantification was

performed using individual calibration curves for each identified compound. The concentrations of volatile compounds were expressed as ng g^{-1}.

Compound identification was performed on an Agilent 6890N gas chromatograph/mass spectrometer (Agilent Technologies, Inc.) using the same capillary column as was used in the GC analyses. Mass spectra were obtained by electron impact ionization at 70 eV. Helium was used as the carrier gas, and the same temperature gradient programme described previously was used for MS acquisition. Spectrometric data were recorded (Hewlett-Packard 3398 GC Chemstation) and compared with those from the original NIST HP59943C library mass spectra.

2.6. Statistical analysis

Prior to ANOVA, cfu g^{-1} data were converted to $\log_{10}$ cfu g^{-1}. Other data were not converted. Data were analysed using general linear model analysis with JMP®8 software (JMP®8, SAS Institute, Cary, NC, USA). After analysis of variance (ANOVA), significant differences between treatments for each sampling time were analysed by Student's t test or Tukey's test at a significance level of $P < 0.05$.

Unscrambler version 9.1.2. Software (CAMO, 2004) was used to develop a partial least square regression (PLSR) model. The PLSR model was used as a predictive method to relate the CPA-7 population (Y) to a set of explanatory variables (X), which include the volatile compound emissions and O_2 and CO_2 concentrations. As a pretreatment, the data were centred and weighted using the inverse of the standard deviation of each variable in order to avoid the influence of the different scales used for the variables (Martens and Naes, 1989). A full cross validation was run as a validation procedure.

3. Results

3.1. Population of microorganisms on fresh-cut pear stored at 5 °C

Initial *S. enterica* populations (Fig. 1A) were approximately 3.40 log cfu g^{-1} regardless $CaCl_2$ treatment and the presence of CPA-7. The *S. enterica* population decreased throughout the storage time (9 days) by more than 0.5-log units, and significant differences were observed between the initial and final values. Neither CPA-7 nor $CaCl_2$ postharvest treatment were found to have an effect against *S. enterica* under the conditions tested.

The initial populations of *L. monocytogenes* were between 2.80 and 3.00-log units after the inoculation of the pear wedges (Fig. 1B). When pears treated with CPA-7 but untreated or treated with $CaCl_2$ were compared, significant differences in the population were reported after 2 and 6 days of storage for both treatments (b and d). The populations of *L. monocytogenes* on fresh-cut pear and pear untreated with CPA-7 increased during the storage time and reached similar values (5.62 ± 0.11 log cfu g^{-1} on $CaCl_2$-treated pear and 5.65 ± 0.15 log cfu g^{-1} on $CaCl_2$-untreated pear wedges). On pear wedges treated with CPA-7, the final *L. monocytogenes* population was not influenced by the $CaCl_2$ treatment and reached values of 4.71 ± 0.22 log cfu g^{-1} on pear wedges treated with $CaCl_2$ and 4.88 ± 0.21 log cfu g^{-1} on untreated pear wedges. CPA-7 significantly reduced (approximately 1-log unit) the population of *L. monocytogenes* after 9 days of storage at 5 ± 1 °C.

Regardless of the postharvest $CaCl_2$ treatment, initial CPA-7 populations (treatment c) (Fig. 1C) were the same (5.59 ± 0.06 and 5.54 ± 0.06 log cfu g^{-1} on pear wedges untreated and treated with $CaCl_2$, respectively). Both populations increased after 9 days of storage and reached 6.61 ± 0.03 and 7.09 ± 0.05 log cfu g^{-1} on pear wedges untreated and treated with $CaCl_2$, respectively. Populations on pear wedges treated with $CaCl_2$ increased faster than populations on fresh-cut pear not treated with $CaCl_2$. Significant

differences were found at 2 and 9 days of storage. Regardless of the CaCl2 postharvest treatment, the population of TAM in pear wedges not treated with CPA-7 did not exceed 3.50 log cfu g^{-1} during the experiment (data not shown).

3.2. Headspace gas concentration

The O_2 concentration decreased from 21.0 kPa to between 12.6 and 14.6 kPa after 9 days of storage (Table 1), and there were no significant differences from the treatments at any of the tested times. The CO_2 concentration increased throughout the storage period until it reached values from 7.8-9.7 kPa. Except at day 2, no significant effects of $CaCl_2$ and CPA-7 treatments were found.

3.3. Soluble solids content (SSC) and titratable acidity (TA)

The SSC ranged from 13.0 to 14.8 % during the assay (Table 2). The SSC values of postharvest $CaCl_2$-treated (CaControl and CaCPA-7) pears were higher than those of the CPA-7-treated pears. In general, the SSCs were also significantly lower for CPA-7-treated fresh-cut pears.

There were not significant differences in the TA prior to the different treatments (Table 2). After 2, 6 and 9 days of storage, the TA values of the $CaCl_2$-untreated pear samples (Control and CPA-7) were similar. There were no significant differences in the TA due to the presence of CPA-7 in the $CaCl_2$-treated pears (CaControl and CaCPA-7). The TA was only influenced by CPA-7 after 9 days of storage in $CaCl_2$-untreated pears; the TA value was significantly lower (1.27 g L^{-1}) for fresh-cut pears treated with CPA-7 than for CPA-7-untreated ones (1.70 g L^{-1}). At the end of the storage period, each TA value was significantly lower than the initial value for all treatments.

3.4. Ethanol and acetaldehyde concentrations

The initial concentrations of ethanol were between 40.8 and 70.6 mL L^{-1}, and no significant differences were observed between treatments (Fig. 2A). After 6 days of

storage, the ethanol concentration was significantly higher in the $CaCl_2$-treated pear samples without CPA-7 (Ca Control, 175.2 mL L^{-1}) than in other treatments. The ethanol concentrations in the fresh-cut pears significantly increased (by a factor of approximately two) during the storage period in all treatments regardless of the presence of CPA-7. Thus, the increase could not be attributed to the biopreservation culture.

The initial concentration of acetaldehyde was between 3.1 and 4.3 mL L^{-1} (Fig. 2B). The acetaldehyde concentration increased throughout storage, reached its maximum levels after 6 days, and then remained constant. No significant differences were observed between treatments at the end of the storage.

3.5. Volatile compound emissions

Tables 3 and 4 show the mean concentrations of the volatile compounds emitted by the pear wedges on the day of the assay (0 days) and after 2 and 6 days of storage at 5 ± 1 °C. A total of 43 compounds (25 esters, 10 alcohols, 4 aldehydes, 1 terpene, 2 ketones and 1 acid) were identified and quantified in the volatile fraction emitted by minimally processed fruit. Differences in the volatile profiles were found both before and after cold storage as a function of the postharvest $CaCl_2$ treatment. Two esters (hexyl butanoate and hexyl 2-methylbutanoate) and one ketone (6-methyl-5-hepten-2-one) were not detected in the volatile profile of the pears treated with calcium chloride (Table 3).

In pears treated with $CaCl_2$ after harvest (Table 3), the storage period and inoculation with CPA-7 influenced the contents of individual volatile compounds. Thus, butyl 2-methylbutanoate and ethyl hexanoate were detected for the first time after 6 days at 5 °C.

Different results were obtained in pears untreated with $CaCl_2$ (Table 4); four esters (ethyl 2-methylbutanoate, 2-methylbutyl-2-methylbutanoate, pentyl-2-methylbutanoate, and ethyl hexanoate) were detected for the first time after 2 days at 5 °C, and 1-pentanol was quantified for the first time after 6 days in CPA-7-inoculated samples.

Throughout the cold storage period, the effects of inoculation with CPA-7 on the volatiles profile was more important in pears that had not been treated with calcium chloride after harvest. Thus, in this case, minimally processed pears showed higher concentrations of 16 volatile compounds (8 esters, 4 alcohols, 3 aldehydes and one terpene) than samples not treated with CPA-7 (Table 4). In contrast, in pear wedges treated with $CaCl_2$ and inoculated with CPA-7 (Table 3) only 3 esters and 1 alcohol (3-methyl -2-butanol) increased significantly after 2 and 6 days at 5 °C.

After 6 days of storage at 5 °C, the $CaCl_2$-treated pear wedges inoculated with CPA-7 showed higher concentrations in 6 of the 43 volatile compounds (16 %) in contrast to the 32 volatile compounds that showed higher concentration (74 %) in the non-inoculated CPA-7 samples (Table 3). This difference was mainly due to lower concentrations of aliphatic esters (except methyl and ethyl acetates, ethyl 2-methylbutanoate and butyl butanoate), alcohols (except 3-methyl-2-butanol), aldehydes, α-farnesene and acetic acid than in the samples not treated with CPA-7. Instead, after 6 days of cold storage at 5°C, the $CaCl_2$-untreated and CPA-7-treated minimally processed pear samples emitted higher amounts of 51 % of the volatile compounds in comparison to 19 % of the compounds in samples not inoculated with CPA-7 (Table 4). This result was due to higher ester concentrations (except ethyl, butyl and hexyl acetates, ethyl 2-methylbutanoate and pentyl 3-methylbutanoate), alcohols (except ethanol, 3-methyl-2-butanol and 1-pentanol), aldehydes (except acetaldehyde), α-farnesene and acetic acid of CPA-7 samples in comparison to the pear wedges not treated with CPA-7.

A partial least square regression (PLSR) model was developed to evaluate possible correlations between the CPA-7 population (*Y variable*) and a set of potentially explanatory variables (*X variables*), which included the concentration of the volatile compounds emitted by pear wedges. Samples from day 0 were excluded of this model to refine the differentiation between the control and pear wedges treated with CPA-7. To carry out the analysis, all samples were included (those treated (Ca) or untreated with

$CaCl_2$ (CK) and the samples with CPA-7 (CPA7) or without CPA-7 (Control), stored at 5 ± 1 °C for 2 and 6 days). Therefore, a PLSR analysis including 8 samples and 43 volatile compounds was performed (Fig. 3). According to this model, up to 98 % of the variability was explained by the emission of volatile compounds. The analysis showed two groups; samples treated with CPA-7 were located on the right side of PC1, which explained 95 % of the total variance, and samples without CPA-7 were located on the left side of PC1 (Fig. 3A). The corresponding loadings plot (Fig. 3B) showed that the samples treated with CPA-7 were associated with high concentrations of 1-hexanol and (Z)-2-hexenyl acetate. There was not a clear influence of the volatile compounds on the differentiation of pear wedges treated or untreated with $CaCl_2$ after harvest.

Fig. 4 shows the regression coefficients for the CPA-7 population vs. the emission of volatile compounds. This figure allowed us to identify those volatile components that were most influenced by the CPA-7 population. The application of CPA-7 was related to the emissions of six esters (methyl acetate, 3-methylbutyl acetate, (Z)-2-hexenyl acetate, 2-methylpropyl butanoate, pentyl acetate, and butyl hexanoate), five alcohols (3-methyl-2-butanol, 1-butanol, 2-methyl-1-butanol, 1-hexanol and (E)-2-hexen-1-ol), one aldehyde (hexanal), and acetone.

4. Discussion

In previous studies (Iglesias et al., 2018), we demonstrated that CPA-7 was effective against *S. enterica* and *L. monocytogenes* on pear wedges at air temperatures of 20, 10 and 5 ± 1 °C and determined the antioxidant solution and film best used for commercial applications. In this work, we have focused on the antagonistic activity of CPA-7 against foodborne pathogens under conditions that simulate commercial applications and how the presence of CPA-7 and the $CaCl_2$ postharvest treatment influences several pear quality parameters, including the contents of several volatile compounds.

After harvest of the fruit, cold storage and a controlled atmosphere are essential for delaying the ripening process. Moreover, postharvest dipping in $CaCl_2$ prior to storage extends the commercial life for both whole and minimally processed fruit (Ortiz et al., 2009; Trentham et al, 2008). Calcium can penetrate fruit flesh through lenticels, but cracks in the cuticle play a significant role in calcium entrance into the fruit (Conway et al., 2002; Ortiz et al., 2009). In general, $CaCl_2$ treatment after harvest did not improve CPA-7 effectiveness against foodborne pathogens evaluated; nevertheless, the CPA-7 population was higher on pear wedges treated with $CaCl_2$ after harvest than it was on untreated samples. Microorganisms need calcium for their development, survival and physiological processes (Corbin et al., 2008). Tiwari et al. (1992) observed that an increase in extracellular Ca^{2+} caused an increase in the growth rate of *Rhizobium melitoti*. In addition, Onoda et al. (2000) demonstrated that in absence of Ca^{2+}, *E. coli* stopped growing and cells became unusual in form and could lyse and die. However, it has been demonstrated that the amount of calcium required for bacteria depends on the growth conditions (Youatt, 1993).

CPA-7 was not observed to have antagonistic activity against *S. enterica* under MAP at 5 ± 1 °C, and no pathogen growth was observed. Similarly, Alegre et al. (2013a) did not observe an antagonistic effect against *Salmonella* on apple wedges. Regarding *L. monocytogenes*, we observed an antagonistic effect from CPA-7 after 9 days of storage at 5 ± 1 °C, and it caused reductions of approximately 1-log unit. Alegre et al. (2013a) also demonstrated an antagonistic effect of CPA-7 against *L. monocytogenes* on apple wedges; however, the effect was greater under air conditions than under MAP; a similar effect was observed by Abadias et al. (2014) for fresh-cut melon. According the review by Siroli et al. (2015a) some biocontrol agents were also able to control spoilage microorganisms naturally present in minimally processed fruits and vegetables. In our work, the effect of CPA-7 on the spoilage microorganisms was not evaluated. No visible

symptoms of microbial spoilage were observed neither in CPA-7 and control fresh-cut pears during the shelf-life (9 days at 5º C), so we could not reach to a clear conclusion.

We observed significant differences in the SSC values of untreated and CPA-7-treated fresh-cut pear regardless of postharvest $CaCl_2$ treatment, except after 6 days of storage in the case of the $CaCl_2$-treated pear wedges. The SSC values of pear wedges treated with CPA-7 were 1 % lower than those of untreated pear, which could be perceived by the consumers as a less sweet taste. Regarding the TA values, significant differences were observed after 6 and 9 days of storage between the $CaCl_2$-treated pear inoculated with CPA-7 and non-inoculated samples. It is known that consumers can perceive differences in the TA if the variation is higher than 0.08 % (Harker et al., 2002). In our case, the differences found after 6 and 9 days of storage were lower than this value and therefore could not be perceived by consumers. Alegre et al. (2013a) and Abadias et al. (2014) did not report significant differences in SSC or TA values among fruit (apple wedges or fresh-cut melon) untreated and treated with CPA-7.

The results showed that ethanol and acetaldehyde production was not affected by the presence of CPA-7. We observed that the concentration of ethanol increased throughout the assay up to 95-179 mL L^{-1} regardless of the treatment. The acetaldehyde concentration reached its highest values after 6-9 days of storage. The fact that both metabolites increased during the storage time regardless of the treatment could indicate that the microorganism did not affect to the biosynthesis of these compounds, and they were produced by the fruit metabolism.

The volatile profile emitted by minimally processed Conference pear stored at 5 °C was determined; esters accounted for more than 57 % of the volatile fraction of Conference wedges both treated and untreated with $CaCl_2$. Esters are known as the most abundant class of compounds observed when using headspace analysis, and they are the volatile compounds that contribute the most to the aroma of intact and fresh-cut pears (Chen et

al., 2006, Bai et al., 2009). The major esters of Conference pear aroma (butyl and hexyl acetates) are predominant in other intact *Pyrus communis* pears including Comice (López et al., 2001), d'Anjou (Argenta et al., 2003), and Barlett (Zlatić et al., 2016), and the esters hare highly correlated with the fruity and characteristic pear aroma (Makkumrai et al., 2014).

The impact of CPA-7 inoculation on the volatile profiles of fresh-cut Conference pears differed depending on the $CaCl_2$ treatment and cold storage time. According to the evaluation of volatile emissions during cold storage, 3 esters and 2 alcohols were only detected in Conference wedges inoculated with CPA-7 and not treated with $CaCl_2$, namely, 3-methylbutyl acetate, butyl hexanoate, butyl propanoate, 1-hexanol and 1-octanol. Previous works have shown that 3-methylbutyl acetate and 1-octanol are present in the volatile emission profiles of intact Comice pears (Makkumrai et al., 2014), butyl hexanoate is present in d'Anjou pears, and butyl propanoate and 1-hexanol are present in intact Conference pears (Rizzolo et al., 2005).

When the data were analysed using a partial least square regression (PLSR) model, we could detect 13 volatile compounds (6 were esters, 5 alcohols, 1 aldehyde and 1 ketone) that were key variables for discriminating the samples in two groups (the control and inoculated with CPA-7 samples). The key compounds were methyl acetate (fruity, ripe, and floral notes), 3-methylbutyl acetate (fruity, banana, sweet pear), pentyl acetate (fruity, banana, pear and apples notes), (Z)-2-hexenyl acetate (fruity odour), 2-methylpropyl butanoate (fruity, sweet, pineapple, apple, and tutti-frutti notes), butyl hexanoate (fruity, pineapple, and ripe fruit notes), 3-methyl-2-butanol (alcoholic, spicy, ethereal, cognac, fruity, fresh odour), 1-butanol (fruity, sweet, banana, fruit juice, and tutti-frutti notes), 2-methyl-1-butanol (wine, onion, fruity, alcoholic, and whisky notes), 1-hexanol (herbal, fatty, and fruity), (E)-2-hexen-1-ol (green leafy, fresh, fatty, grassy with fruity and juicy nuances), hexanal (green, woody, vegetative, apple, grassy, citrus and orange with a fresh, lingering aftertaste) and acetone (fruity, blueberry, raspberry, and

berry notes). Among the mentioned compounds, there are some that are common in the volatile profiles of pears (methyl acetate, 3-methylbutyl acetate, 1-butanol, 1-hexanol, and hexanal); therefore, increases in their contents could enhance flavour consumer's perception. Nevertheless, we were not able to carry out a consumer preference test as this strain is not yet included in the QPS (Qualified Presumption of Safety) list of the EFSA.

5. Conclusions

To conclude, CPA-7 was able to control the growth of *L. monocytogenes* after 9 days of storage. On the other hand, no effect was observed on the *S. enterica* population under the tested conditions. These results suggested that CPA-7 did not have a bactericidal effect against foodborne pathogens. CPA-7 treatment could improve the volatile profile and did not negatively affect the fruit quality. We did not observe a clear effect of postharvest $CaCl_2$ treatment on the efficacy of CPA-7, and we studied the quality parameters of fresh-cut pear.

Acknowledgements

Authors are grateful to the Spanish Ministry of Science and Innovation (AGL-2012-38671 research project), Secretaria d'Universitats i Recerca del Departament d'Economia i Coneixement de la Generalitat de Catalunya (AGAUR fellowship 2015-FI-B100156 (M. Belén Iglesias) and 2017-FI-B200164 (Lorena Zudaire)), European Regional Development Fund (FEDER), and CERCA Programme (Generalitat de Catalunya) for their financial support. We also thank Patricia Gálvez and Mariona Guardiola for their technical support.

References

Abadias, M., Altisent, R., Usall, J., Torres, R., Oliveira, M., Viñas, I., 2014. Biopreservation of fresh-cut melon using the strain *Pseudomonas graminis* CPA-7. Postharvest Biol. Technol. *96*, 69-77.

Abadias, M., Usall, J., Anguera, M., Solsona, C., Viñas, I., 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. Int. J. Food Microbiol. 123, 121-129.

Alegre, I., Viñas, I., Usall, J., Anguera, M., Abadias, M., 2011. Microbiological and physicochemical quality of fresh-cut apple enriched with the probiotic strain *Lactobacillus rhamnosus* GG. Food Microbiol. 28, 59-66.

Alegre, I., Viñas, I., Usall, J., Anguera, M., Altisent, R., Abadias, M., 2013a. Antagonistic effect of *Pseudomonas graminis* CPA-7 against foodborne pathogens in fresh-cut apples under simulated commercial conditions. Food Microbiol. 33, 139-148.

Alegre, I., Viñas, I., Usall, J., Teixido, N., Figge, M.J., Abadias, M., 2013b. Control of foodborne pathogens on fresh-cut fruit by a novel strain of *Pseudomonas graminis*. Food Microbiol. 34, 390-399.

Argenta, L.C., Fan, X., Mattheis, J.P. 2003. Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by d'Anjou cv. Pear fruit. J. Agric. Food Chem. 51: 3858-3864.

Bai, J., Wu, P., Manthey, J., Goodner, K., Baldwin, E. 2009. Effect of harvest maturity on quality of fresh-cut pear salad. Postharvest Biol. Technol. 51: 250-256.

CAMO, 2004. Unscrambler Users Guide, ver. 9.1.2. Programme Package for Multivariate Calibration. Trondheim, Norway.

CDC (Centers for Disease Control and Prevention), 2016. List of Selected Multistate Foodborne Outbreak Investigations 2006-2016. Available at: http://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html (accessed 23.01.2016).

Chen, J.L., Yan, S., Feng, Z., Xiao, L., Hu, X.S. 2006. Changes in the volatile compounds and chemical and physical properties of Yali pear (Pyrus bertscheneideri Reld) during storage. Food Chem. 97: 248-255.

Conway, W.S., Sams, C.E., Hickey, K.D., 2002. Pre- and postharvest calcium treatment of apple fruit and its effect on quality. Acta Horticulturae 594, 413-419.

Corbin, B.D., Seeley, E.H., Raab, A., Feldmann, J., Miller, M. R., Torres, V.J., Anderson, K.L., Dattilo, B.M., Dunman, P.M., Gerads, R., Caprioli, R.M., Nacken, W., Chazin, W.J., Skaar, E.P., 2008. Metal chelation and inhibition of bacterial growth in tissue abscesses. Science 319, 962-965.

Echeverría, G., Graell, J., López, M. L., Lara, I., 2004. Volatile production, quality and aroma-related enzyme activities during maturation of 'Fuji' apples. Postharvest Biol. Technol. 31, 217-227.

Glenn, G.M.; Poovaiah, B.W., 1990. Calcium-mediated postharvest changes in texture and cell wall structure and composition in 'Golden Delicious' apples. J. Am. Soc. Hortic. Sci. 1990, 115 (6), 962-968.

Goldberg, I., 1984. Functional Foods, Designer Foods, Pharmafoods, Nutraceuticals. Chapman and Hall, New York.

Harker, F.R., Marsh, K.B., Young, H., Murray, S.H., Gunson, F.A., Walker, S.B., 2002. Sensory interpretation of instrumental measurements 2: sweet and acid taste of apple fruit. Postharvest Biol. Technol. 24 (3), 241-250.

Iglesias, M.B., Abadias, M., Anguera, M., Sabata, J., Viñas, I., 2017. Antagonistic effect of probiotic bacteria against foodborne pathogens on fresh-cut pear. LWT - Food Sci. Technol. 81, 243-249.

Iglesias, M.B., Abadias, M., Anguera, M., Viñas, I., 2018. Efficacy of *Pseudomonas graminis* CPA-7 against *Salmonella* spp. and *Listeria monocytogenes* on fresh-cut pear and setting up of the conditions for its commercial application. Food Microbiol. 87, 581-588.

JMP® 8 User's Guide, 2004. SAS Institute, Inc., Cary, NC.

Lakakul, R., Beaudry, R.M., Hernandez, R.J., 1999. Modeling respiration of apple slices in modified-atmosphere packages. J. Food Sci. 64, 105-110.

Leverentz, B., Conway, W.S., Janisiewicz, W.J., Abadias, M., Kurtzman, C.P., Camp, M.J., 2006. Biocontrol of the food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* serovar Poona on fresh-cut apples with naturally occurring bacterial and yeast antagonists. *Appl. Environ.* Microbiol. 72, 1135-1140.

López, M.L., Miró, R., Graell, J., 2001. Quality and aroma production of Doyenne du Comice pears in relation to harvest date and storage atmosphere. Food Sci. Technol. Int. 7, 493-500.

Makkumrai, W., Anthon, G.E., Sivertsen, H., Ebeler, S.E., Negre-Zakharov, F., Barrett, D.M., Mitcham, E.J. 2014. Effect of ethylene and temperature conditioning on sensory attributes and chemical composition of 'Bartlett' pears. Postharvest Biol. Technol. 97: 44-61.

Martens H., Naes T., 1989. *Partial least squares regression. Multivariate calibration*. Chichester: J. Wiley & Sons, Inc.

Onoda, T., Enokizono, J., Kaya, H., Oshima, A., Freestone, P., Norris, V. 2000. Effects of calcium and calcium chelators on growth and morphology of *Escherichia coli* L-form NC-7. J. Bacteriol.182, 1419-1422.

Ortiz, A., Echeverria, G., Graell, J., Lara, I., 2009. Calcium dips enhance volatile emission of cold-stored 'Fuji Kiku-8' apples. J. Agri. Food Chem. 57, 4931-4938.

Parish, M.E., Beuchat, L.R., Suslow, T.V., Harris, L.J., Garrett, E.H., Farber, J.N., Busta, F.F., 2003. Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. Compr. Rev. Food Sci. Food Saf. 2, 161-173.

Qin, G., Tao, S., Cao, Y., Wu, J., Zhang, H., Huang, W., Zhang, S., 2012. Evaluation of the volatile profile of 33 *Pyrus ussuriensis* cultivars by HS-SPME with GC-MS. Food Chem.134, 2367-2382.

Rizzolo, A., Cambiaghi, P., Grassi, M., Zerbini, P.E., 2005. Influence of 1-methylcyclopropene and storage atmosphere on changes in volatile compounds and fruit quality of conference pears. J. Agri. Food Chem. 53, 9781-9789.

Rodriguez-Aguilera, R., Oliveira, J.C., Montanez, J.C., Mahajan, P.V., 2009. Gas exchange dynamics in modified atmosphere packaging of soft cheese. J. Food Engin. 95, 438-445.

Rosen, J.C., Kader, A.A., 1989. Postharvest physiology and quality maintenance of sliced pear and strawberry fruits. J. Food Sci. 54, 656-659.

Sapers, G.M., Miller, R.L., 1998. Browning inhibition in fresh-cut pears. J. Food Sci. 63, 342-346.

Siroli, L., Patriagnani, F., Serrazanetti, D.I., Gardini, F., Lanciotti, R., 2015a. Innovative strategies based on the use of bio-control agents to improve the safety, shelf-life and quality of minimally processed fruits and vegetables. Trends Food Sci. Technol. 46, 302-310.

Siroli, L., Patrignani, F., Serrazanetti, D.I., Tabanelli, G., Montanari, C., Gardini, F., Lanciotti, R., 2015b. Lactic acid bacteria and natural antimicrobials to improve the safety and shelf-life of minimally processed sliced apples and lamb's lettuce. Food Microbiol. 47, 74-84.

Soliva-Fortuny, R.C., Alos-Saiz, N., Espachs-Barroso, A., Martin-Belloso, O., 2004. Influence of maturity at processing on quality attributes of fresh-cut conference pears. J. Food Sci. 69, 290-294.

Soliva-Fortuny, R.C., Oms-Oliu, G., Martin-Belloso, O., 2002. Effects of ripeness stages on the storage atmosphere, color, and textural properties of minimally processed apple slices. J. Food Sci. 67, 1958-1963.

Tiwari, R.P., Reeve, W.G., Gleenn, A.R., 1992. Mutations conferring acid sensitivity in the acid-tolerant strains *Rhizobium meliloti* WSM419 and *Rhizobium leguminosarum* biovar viciae WSM710. FEMS Microbiol. Lett. 100, 107-112.

Toivonen, P.M.A., 2006. Fresh-cut apples: challenges and opportunities for multidisciplinary research. Can. J. Plant Sci. 86, 1361-1368.

Toivonen, P.M.A., Delaquis, P., 2006. Low-volume sprays to treat fresh-sliced apples with anti-browning solution. HortTechnology 16, 257–261.

Trentham, W.R., Sams, C.E., Conway, W.S., 2008. Histological effects of calcium chloride in stored apples. J. Am. Soc. Hort. Sci.133, 487-491.

Trias, R., Baneras, L., Badosa, E., Montesinos, E., 2008. Bioprotection of Golden Delicious apples and Iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. Int. J. Food Microbiol. 123, 50-60.

Wang, X., Ouyang, Y., Liu, J., Zhu, M., Zhao, G., Bao, W., Hu, F.B., 2014. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. Brit. Med.J. 349.

Youatt, J., 1993. Calcium and microorganisms. Crit. Rev.Microbiol. 19, 83-97.

Zlatić, E., Zadnik, V., Fellman, J., Demšar, Hribar, J., Željko, C., Vidrih, R. 2016. Comparative analysis of aroma compounds in ‘Bartlett’ pear in relation to harvest date, storage conditions and shelf-life. Postharvest Biol. Technol. 117:71-80.

Table 1. Headspace gas composition (O_2 and CO_2, KPa) inside fresh-cut pear trays stored at 5 °C ± 1 °C treated (Ca) or not (None) with 1 % $CaCl_2$ after harvest and inoculated (CPA-7) or not (Control) with 10^8 cfu mL^{-1} of *P. graminis* CPA-7 after cutting. Different capital letters in the same row indicate significant differences during storage time according to a Tukey test (P<0.05) and different lowercase letters in the same column indicate significant differences between different treatment at the same time sampling for each gas according to a Tukey test (P<0.05).

	Postharvest treatment	Biopreservation treatment	Days			
			0	2	6	9
O_2	None	None (Control)	21.0 ± 0.0 Ax	14.7 ± 0.5Bx	15.6 ± 1.4 Bx	14.6 ± 0.1 Bx
		CPA-7 (CPA-7)	21.0 ± 0.0 Ax	15.8 ± 0.6 Bx	14.7 ± 0.2 Bx	14.3 ± 1.0 Bx
	$CaCl_2$	None (CaControl)	21.0 ± 0.0 Ax	14.8 ± 1.9 Bx	16.4 ± 1.4 Cx	12.6 ± 1.0 Cx
		CPA-7 (CaCPA7)	21.0 ± 0.0 Ax	15.7 ± 0.4 Bx	15.0 ± 1.6 Bx	13.0 ± 2.0 Bx
CO_2	None	None (Control)	0.0 ± 0.0 Bx	6.1 ± 0.2 Ax	6.6 ± 1.4 Ax	7.9 ± 0.1 Ax
		CPA-7 (CPA-7)	0.0 ± 0.0 Cx	4.9 ± 0.4 By	7.1 ± 0.3 Ax	7.8 ± 0.9 Ax
	$CaCl_2$	None (CaControl)	0.0 ± 0.0 Cx	5.7 ± 0.7 Bxy	5.9 ± 1.5 Bx	9.7 ± 0.8 Ax
		CPA-7 (CaCPA7)	0.0 ± 0.0 Cx	4.7 ± 0.1 By	7.0 ± 1.4 ABx	9.3 ± 1.8 Ax

Table 2. Solids soluble content (SSC, %) and titratable acidity (TA, g L^{-1}) produced on fresh-cut pear stored at 5 °C treated (Ca) or not (None) with 1 % $CaCl_2$ after harvest and inoculated (CPA-7) or not (Control) with 10^8 cfu mL^{-1} of *P. graminis* CPA-7 after cutting. Different capital letters in the same row indicate significant differences within the same treatment along the storage time according to Tukey's test ($P < 0.05$). Different lower case letters in the same column indicate significant differences between treatments at each sampling time according to Tukey's test ($P < 0.05$).

	Postharvest treatment	Biopreservation treatment	Days at 5ºC			
			0	2	6	9
SSC (%)	None	None (Control)	13.9 ± 0.0 Cb	14.0 ± 0.1 ABb	13.9 ± 0.1 BCb	14.1 ± 0.1 Ac
		CPA-7 (CPA-7)	13.0 ± 0.0 Cc	13.7 ± 0.1 Ac	13.4 ± 0.1 Bc	13.1 ± 0.1 Cd
	$CaCl_2$	None (CaControl)	14.5 ± 0.1 Ba	14.5 ± 0.1 Ba	14.2 ± 0.1 Ca	14.8 ± 0.0 Aa
		CPA (CaCPA-7)	13.9 ± 0.0 Bb	14.2 ± 0.1 Ab	14.3 ± 0.0 Aa	14.3 ± 0.1 Ab
TA (g L^{-1})	None	None (Control)	1.99 ± 0.07 Aa	2.10 ± 0.11 Aa	1.70 ± 0.08 Bab	1.70 ± 0.07 Ba
		CPA-7 (CPA-7)	1.95 ± 0.08 Aa	2.14 ± 0.18 Aa	1.89 ± 0.08 Aa	1.27 ± 0.10 Bb
	$CaCl_2$	None (CaControl)	1.99 ± 0.05 Aa	1.94 ± 0.12 Aa	1.63 ± 0.08 Bb	1.60 ± 0.03 Ba
		CPA-7 (CaCPA-7)	1.83 ± 0.13 Aba	1.94 ± 0.14 Aa	1.62 ± 0.05 Bb	1.58 ± 0.04 Ba

Table 3. Volatile compounds (ng g^{-1}) produced by minimally processed pear stored at 5 °C treated with $CaCl_2$ after harvest. Different capital letters indicate significant differences between pear wedges treated and untreated with CPA-7 the same sampling time according to Student's t test at significance level of $P < 0.05$. nd: not detected. traces: ≤ 10 ng g^{-1}

Volatile compounds	Treated with $CaCl_2$					
	0 days		2 days		6 days	
	CPA-7	no CPA-7	CPA-7	no CPA-7	CPA-7	no CPA-7
ACETATES						
Methyl acetate	899,9 A	106,7 B	107,6 A	nd	1379,2 A	nd
Ethyl acetate	2813,8 A	1388,5 B	2488,7 A	2122,8 B	1172,7 A	707,5 B
Propyl acetate	127,7 B	296,8 A	251,8 B	449,0 A	492,4 B	3538,6 A
Butyl acetate	4746,0 B	13545,4 A	10425,1 B	22699,3 A	9860,3 A	4635,0 B
3-Methylbutyl acetate	nd	417,3 A	144,3 B	211,8 A	168,7 B	1589,4 A
Pentyl acetate	226,0 B	356,8 A	156,8 A	traces B	538,4 B	10044,7 A
Hexyl acetate	6266,3 B	6414,3 A	4872,9 B	7838,3 A	8269,8 B	13127,6 A
(Z)-2-hexenyl acetate	271,8 A	traces B	188,8 A	traces B	87,4 B	1943,1 A
Octyl acetate	532,3 A	46,3 B	619,0 A	64,7 B	164,6 B	1166,8 A
BUTANOATES						
Methyl butanoate	433,1 A	89,2 B	372,8 A	nd	131,9 B	2742,5 A
Ethyl 2-methylbutanoate	58,4 B	83,6 A	85,0 A	nd	8789,8 A	1931,7 B
2-Methylpropyl butanoate	246,5 B	525,8 A	664,9 A	662,1 A	596,0 B	2105,9 A
Butyl 2-methylbutanoate	nd	nd	nd	nd	158,5 B	1558,2 A
Butyl butanoate	70,1 A	71,6 A	154,1 B	242,0 A	207,1 A	134,5 B
2-Methylbutyl-2-methylbutanoate	149,6 B	357,1 A	nd	416,8 A	nd	5884,4 A
Hexyl butanoate	nd	nd	nd	nd	nd	nd
Hexyl 2-methylbutanoate	nd	nd	nd	nd	nd	nd
HEXANOATES						
Ethyl hexanoate	nd	nd	nd	nd	782,1 B	2791,0 A
Butyl hexanoate	475,2 A	nd	1235,3 B	1471,1 A	604,4 B	7734,6 A
Pentyl hexanoate	116,6 A	nd	190,4 A	nd	nd	1097,6 A
Hexyl hexanoate	392,9 A	334,7 A	280,3 A	nd	188,8 B	2696,4 A
PROPANOATES						
tert-Butyl propanoate	137,5 A	97,7 B	94,5 A	nd	256,7 B	2198,5 A
Butyl propanoate	148,2 A	45,9 B	238,6 A	nd	89,9 B	1759,6 A
OCTANOATES						
Ethyl octanoate	956,6 A	nd	287,4 A	67,0 B	nd	815,1 A
PENTANOATES						
Pentyl 3-methylbutanoate	nd	179,2 A	nd	236,8 A	nd	1800,1 A
ALCOHOLS						
Ethanol	24582,3 B	61475,7 A	6170,3 B	8099,1 A	2971,8 B	11412,8 A
3-Methyl-2-butanol	11972,9 B	14742,8 A	11672,9 A	nd	12480,4 A	4561,2 B
1-Butanol	59,1 B	81,6 A	75,4 A	nd	105,4 B	2729,8 A
2-Methyl-1-butanol	nd	73,1 A	nd	nd	205,0 B	731,9 A
1-Pentanol	166,5 B	278,0 A	nd	109,3 A	60,6 B	1588,3 A
1-Hexanol	233,0 A	nd	304,5 A	nd	261,3 B	1122,5 A
(E)-2-Hexen-1-ol	traces B	396,1 A	traces A	traces A	traces A	traces A
2-Ethyl-1-hexanol	3674,8 A	1197,0 B	3439,4 A	723,7 B	793,4 B	9456,4 A
1-Octanol	94,8 A	59,0 B	181,1 A	75,4 B	nd	170,7 A
Benzyl alcohol	2809,1 A	329,7 B	6167,3 A	664,0 B	304,3 B	10877,9 A
ALDEHYDES						
Acetaldehyde	1139,8 A	985,5 B	673,4 A	nd	nd	693,4 A
Hexanal	714,0 A	594,1 B	37316,8 A	2546,8 B	1713,9 B	4962,6 A
2-Ethylhexanal	52,4 B	260,2 A	78,0 B	1127,0 A	69,3 B	1048,3 A
Benzaldehyde	352,7 A	nd	489,1 A	202,8 B	201,2 B	1402,2 A
TERPENES						
α-Farnesene	577,4 A	50,6 B	583,3 A	135,9 B	776,9 B	1188,2 A
KETONES						
Acetone	531,4 A	traces B	traces A	traces A	traces A	traces A
6-Methyl-5-hepten-2-one	nd	nd	nd	nd	nd	nd
ACIDS						
Acetic acid	252,5 A	nd	583,8 A	90,1 B	nd	2905,3 A

644

Table 4. Volatile compounds produced (ng g^{-1}) by minimally processed pear stored at 5 °C untreated with $CaCl_2$ after harvest. Different capital letters indicate significant differences between pear wedges treated and untreated with CPA-7 the same sampling time according to Student's t test at significance level of $P < 0.05$. nd: not detected. traces: ≤ 10 ng·g^{-1}

Volatile compounds	Untreated with $CaCl_2$					
	0 days		2 days		6 days	
	CPA-7	no CPA-7	CPA-7	no CPA-7	CPA-7	no CPA-7
ACETATES						
Methyl acetate	258,0 A	107,0 B	traces A	traces A	traces A	traces A
Ethyl acetate	609,3 B	1347,3 A	1520,5 B	1649,0 A	782,0 B	2628,8 A
Propyl acetate	315,9 A	395,7 A	556,5 A	503,1 A	484,8 A	289,0 B
Butyl acetate	9768,8 A	5127,2 B	18653,7 A	18972,4 A	13518,8 B	14022,1 A
3-Methylbutyl acetate	237,7 A	nd	274,2 A	nd	187,5 A	nd
Pentyl acetate	nd	17,8 A	nd	nd	nd	nd
Hexyl acetate	8301,7 A	5043,2 B	11384,6 A	6844,1 B	5776,1 B	7945,7 A
(Z)-2-hexenyl acetate	351,5 A	traces B	292,8 A	traces B	245,9 A	traces B
Octyl acetate	1348,6 A	332,7 B	173,1 A	nd	259,5 A	nd
BUTANOATES						
Methyl butanoate	187,3 A	nd	68,6 B	224,4 A	132,1 A	nd
Ethyl 2-methylbutanoate	nd	nd	367,8 A	nd	nd	185,1 A
2-Methylpropyl butanoate	704,4 A	115,4 B	824,5 A	753,9 B	933,9 A	487,3 B
Butyl 2-methylbutanoate	nd	nd	nd	nd	nd	nd
Butyl butanoate	230,9 A	traces B	385,1 A	traces B	558,9 A	407,2 B
2-Methylbutyl-2-methylbutanoate	nd	nd	nd	303,1 A	nd	nd
Hexyl butanoate	373,6 A	nd	nd	nd	nd	nd
Hexyl 2-methylbutanoate	577,3 A	nd	nd	nd	nd	nd
HEXANOATES						
Ethyl hexanoate	nd	nd	123,4 A	nd	nd	nd
Butyl hexanoate	334,8 A	nd	1125,9 A	nd	920,9 A	nd
Pentyl hexanoate	478,3 A	nd	nd	nd	nd	nd
Hexyl hexanoate	1006,4 A	599,7 B	466,8 A	254,0 B	296,5 A	nd
PROPANOATES						
tert-Butyl propanoate	nd	115,7 A	nd	nd	nd	nd
Butyl propanoate	222,1 A	nd	116,8 A	nd	331,4 A	nd
OCTANOATES						
Ethyl octanoate	437,1 A	nd	nd	119,6 A	51,0 A	nd
PENTANOATES						
Pentyl 3-methylbutanoate	nd	nd	87,9 A	nd	nd	406,4 A
ALCOHOLS						
Ethanol	188734,4 A	3676,9 B	13305,8 A	6648,5 B	1983,8 B	6586,0 A
3-Methyl-2-butanol	221,3 B	15256,4 A	391,2 A	nd	nd	321,6 A
1-Butanol	nd	118,9 A	nd	nd	nd	nd
2-Methyl-1-butanol	nd	nd	114,2 A	nd	nd	nd
1-Pentanol	nd	nd	nd	nd	nd	122,2 B
1-Hexanol	291,0 A	nd	406,8 A	nd	368,7 A	nd
(E)-2-Hexen-1-ol	traces A	traces A	traces A	traces A	767,0 A	traces B
2-Ethyl-1-hexanol	5575,7 A	2445,7 B	1141,2 A	516,4 B	1133,4 A	304,1 B
1-Octanol	506,4 A	104,4 B	182,4 A	nd	106,0 A	nd
Benzyl alcohol	7283,4 A	172,5 B	2685,9 A	1239,0 B	4129,8 A	442,4 B
ALDEHYDES						
Acetaldehyde	2122,5 A	780,4 B	401,4 B	834,5 A	nd	1262,8 A
Hexanal	1521,9 A	nd	2813,0 A	1381,0 B	6668,4 A	1515,8 B
2-Ethylhexanal	56,3 A	59,3 A	156,1 A	88,9 B	110,9 A	78,4 B
Benzaldehyde	1123,0 A	649,4 B	399,8 A	nd	405,5 A	nd
TERPENES						
α-Farnesene	3658,5 A	931,7 B	264,6 A	traces B	531,9 A	traces B
KETONES						
Acetone	traces B	62,9 A	traces A	traces A	traces A	traces A
6-Methyl-5-hepten-2-one	405,3 A	nd	119,3 A	nd	nd	nd
ACIDS						
Acetic acid	775,9 A	nd	nd	nd	164,9 A	nd

FIGURE CAPTIONS

Fig. 1 *Salmonella* (A), *L. monocytogenes* (B) and CPA-7 (C) population (log cfu g^{-1}) on fresh-cut pear treated or not with $CaCl_2$ 1 % after harvest and then processed and stored at 5 ± 1 °C. The results are the means of three values. Vertical bars indicate the standard deviations of the means. Different capital letters indicate significant differences within the same treatment throughout the storage time according to Tukey's test ($P < 0.05$). Different lower-case letters indicate significant differences among the same treatment on pears untreated or treated with $CaCl_2$ at each sampling time according to Student's t test ($P < 0.05$). * Indicates significant differences between samples with or without CPA-7 at each sampling time (Student's t test at significance level of $P < 0.05$).

Fig. 2 Concentration ($mL \cdot L^{-1}$) of ethanol (A) and acetaldehyde (B) produced on $CaCl_2$-untreated pear wedges inoculated without CPA-7 or with CPA-7 and $CaCl_2$-treated pear wedges inoculated without CPA-7 or with CPA-7 processed and stored at 5 ± 1 °C. The results are the means of 3 values. Vertical bars indicate the standard deviations of the means. Different capital letters indicate significant differences within the same treatment along the storage time according to Tukey's test ($P < 0.05$). Different lower-case letters indicate significant differences between treatments at each sampling time according to Tukey's test ($P < 0.05$).

Fig. 3 Score (A) and loading (B) plots of PC1 vs. PC2 corresponding to a PLSR model for CPA-7 population vs. emissions of volatile compounds on pear wedges stored at 5° C.

Fig. 4 Regression coefficients corresponding to a PLSR model for CPA-7 population vs. emissions of volatile compounds on pear wedges stored at 5 ± 1 °C.

Figure 1

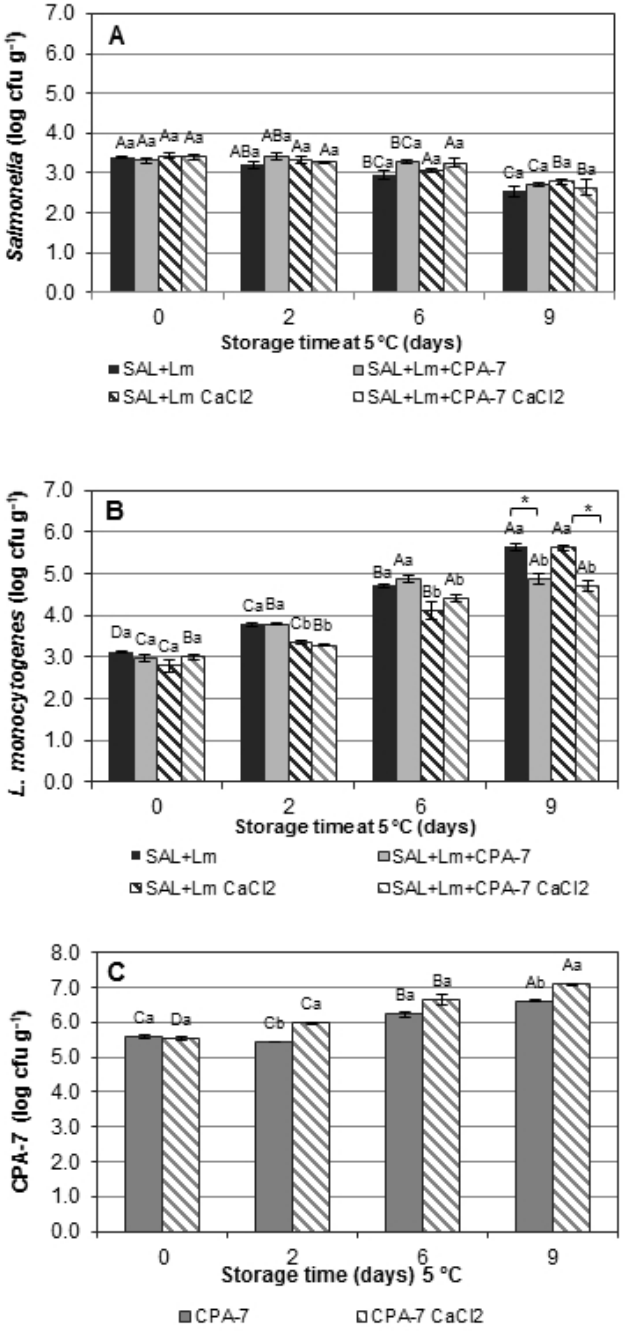
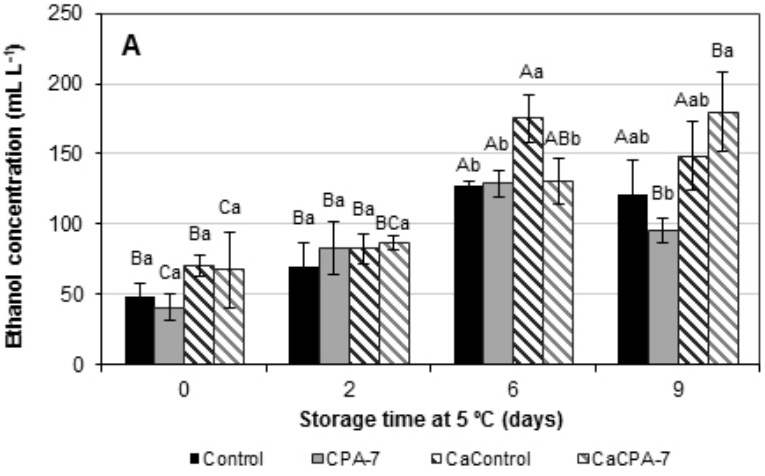

A
Salmonella (log cfu g-1)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
AaAa Aa Aa
ABa ABa Aa Aa
BCa BCa Aa Aa
Ca Ca Ba Ba
0
2
6
9
Storage time at 5 °C (days)
SAL+Lm
SAL+Lm+CPA-7
SAL+Lm CaCl2
SAL+Lm+CPA-7 CaCl2
B
L. monocytogenes (log cfu g-1)
Da Ca Ca Ba
Ca Ba Cb Bb
Ba Aa Bb Ab
Aa Ab Aa Ab
*
*
Storage time at 5 °C (days)
C
CPA-7 (log cfu g-1)
8.0
Ca Da
Cb Ca
Ba Ba
Ab Aa
Storage time (days) 5 °C
CPA-7
CPA-7 CaCl2

Figure 2


A
Ethanol concentration (mL L-1)
250
200
150
100
50
0
Ba
Ca
Ba
Ca
Ba
Ba
Ba
BCa
Ab
Ab
Aa
ABb
Aab
Bb
Aab
Ba
0
2
6
9
Storage time at 5 ºC (days)
Control
CPA-7
CaControl
CaCPA-7

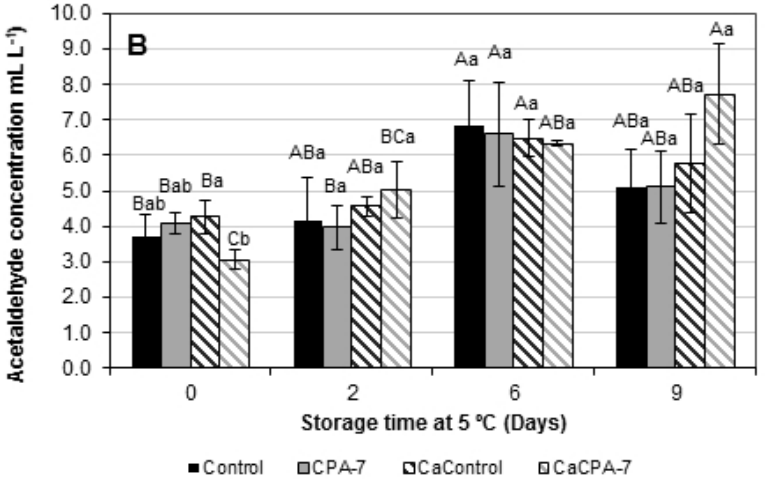
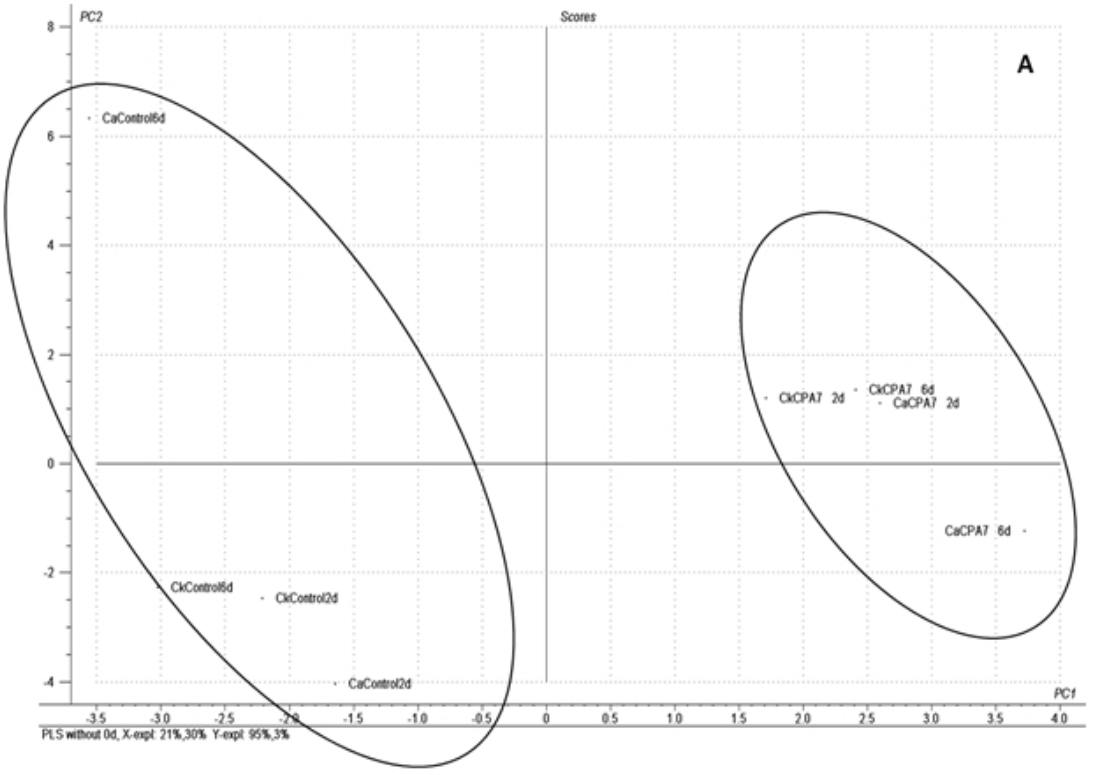
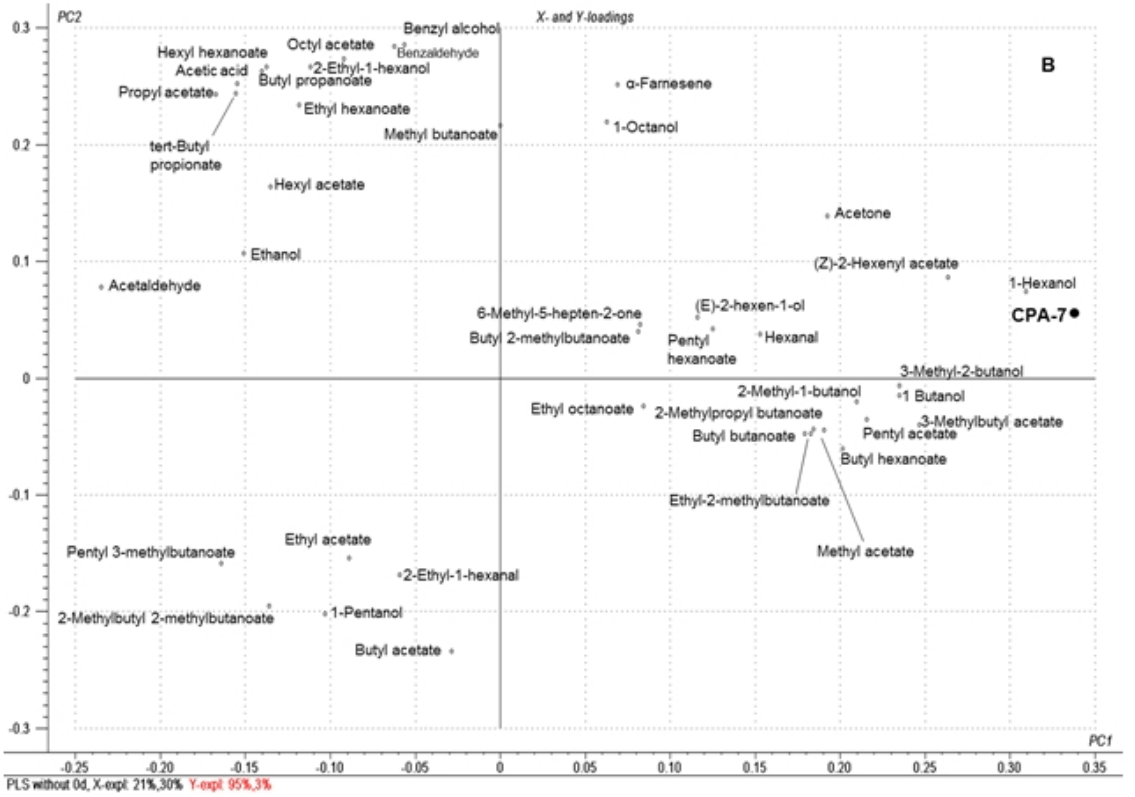

B
Acetaldehyde concentration mL L-1)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Bab
Bab
Ba
Cb
ABa
Ba
ABa
BCa
Aa
Aa
Aa
ABa
ABa
ABa
ABa
Aa
0
2
6
9
Storage time at 5 ºC (Days)
Control
CPA-7
CaControl
CaCPA-7

Figure 3


PC2
Scores
A
CaControl6d
CkCPA7 2d
CkCPA7 6d
CaCPA7 2d
CaCPA7 6d
CkControl6d
CkControl2d
CaControl2d
PC1
PLS without 0d, X-expl: 21%,30% Y-expl: 95%,3%


PC2
X- and Y-loadings
B
Benzyl alcohol
Benzaldehyde
Octyl acetate
Hexyl hexanoate
Acetic acid
2-Ethyl-1-hexanol
Butyl propanoate
Propyl acetate
Ethyl hexanoate
Methyl butanoate
tert-Butyl propionate
Hexyl acetate
Ethanol
Acetaldehyde
α-Farnesene
1-Octanol
Acetone
(Z)-2-Hexenyl acetate
1-Hexanol
CPA-7
(E)-2-hexen-1-ol
6-Methyl-5-hepten-2-one
Butyl 2-methylbutanoate
Pentyl hexanoate
Hexanal
3-Methyl-2-butanol
2-Methyl-1-butanol
1 Butanol
Ethyl octanoate
2-Methylpropyl butanoate
3-Methylbutyl acetate
Pentyl acetate
Butyl butanoate
Butyl hexanoate
Ethyl-2-methylbutanoate
Methyl acetate
Ethyl acetate
Pentyl 3-methylbutanoate
2-Ethyl-1-hexanal
2-Methylbutyl 2-methylbutanoate
1-Pentanol
Butyl acetate
PC1
PLS without 0d, X-expl: 21%,30% Y-expl: 95%,3%

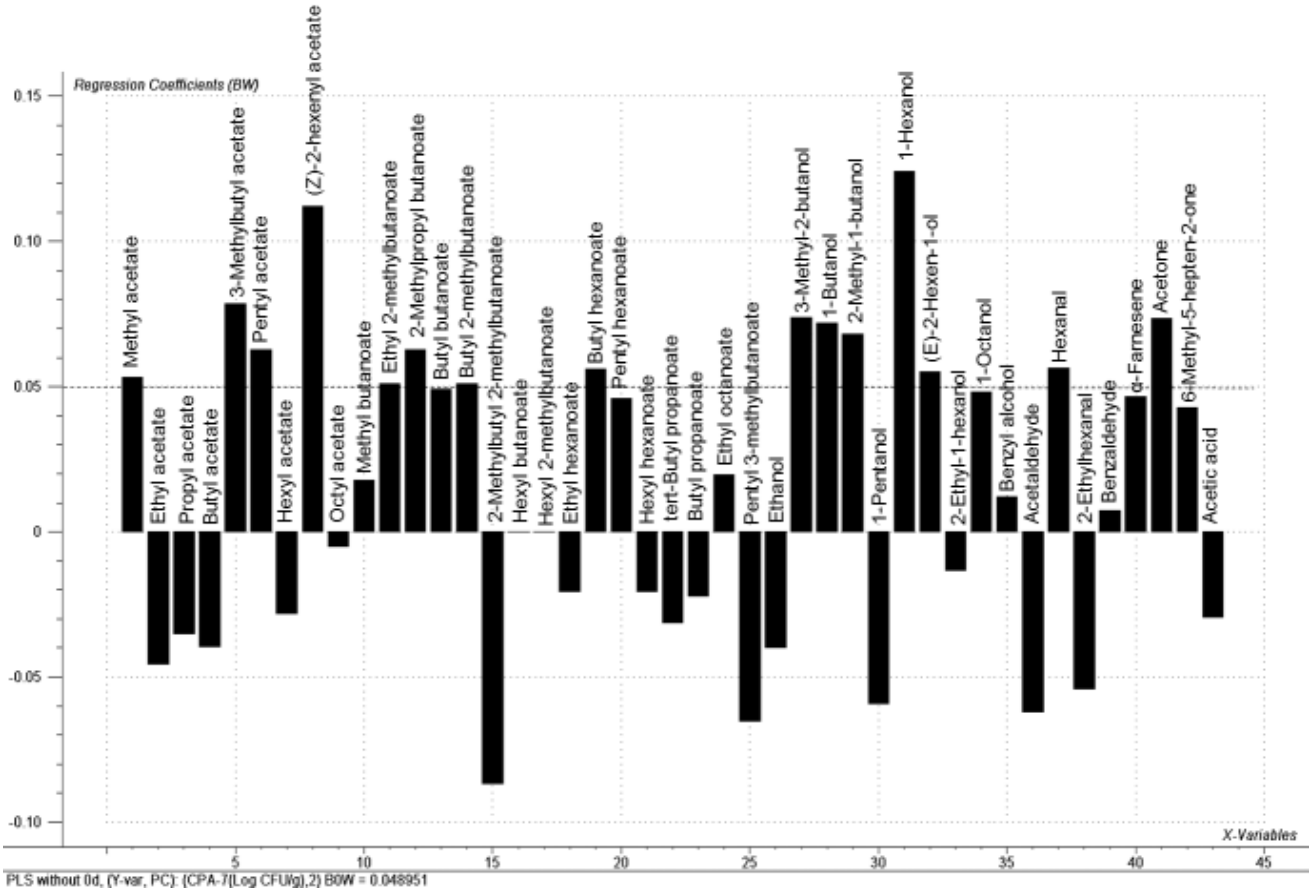

Regression Coefficients (BW)
0.15
0.10
0.05
0
-0.05
-0.10
Methyl acetate
Ethyl acetate
Propyl acetate
Butyl acetate
3-Methylbutyl acetate
Pentyl acetate
Hexyl acetate
(Z)-2-hexenyl acetate
Octyl acetate
Methyl butanoate
Ethyl 2-methylbutanoate
2-Methylpropyl butanoate
Butyl butanoate
Butyl 2-methylbutanoate
2-Methylbutyl 2-methylbutanoate
Hexyl butanoate
Hexyl 2-methylbutanoate
Ethyl hexanoate
Butyl hexanoate
Pentyl hexanoate
Hexyl hexanoate
tert-Butyl propanoate
Butyl propanoate
Ethyl octanoate
Pentyl 3-methylbutanoate
Ethanol
3-Methyl-2-butanol
1-Butanol
2-Methyl-1-butanol
1-Pentanol
1-Hexanol
(E)-2-Hexen-1-ol
2-Ethyl-1-hexanol
1-Octanol
Benzyl alcohol
Acetaldehyde
Hexanal
2-Ethylhexanal
Benzaldehyde
α-Farnesene
Acetone
6-Methyl-5-hepten-2-one
Acetic acid
X-Variables
5
10
15
20
25
30
35
40
45
PLS without 0d, {Y-var, PC}: {CPA-7(Log CFU/g),2} B0W = 0.048951

Figure 4